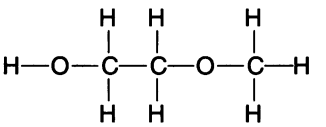
The Use of Rats in the RACB Protocol

Ethylene Glycol Monomethyl Ether: Litter Five



CAS #109-86-4
Sprague-Dawley rats, at 0.0, 0.006, .012, .024%, drinking water
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Reproductive Assessment by Continuous Breeding (RACB) studies were originally designed to be conducted using mice, with the initial justification relating to their size and relative cost (Gulati et al., Fundam Appl Toxicol 17:270–279 [1991]). However, the rat is more commonly used for investigative and regulatory studies. There should be no *a priori* reason why the rat could not be used in a design like the RACB: the two species would appear to share more similarities than differences.

However, one potential problem for the RACB is that when a compound is toxic to reproduction, it often results in sterile matings (or no matings) toward the end of the cohabitation period, which severely reduces the number of offspring available to test in a second generation. A way around this difficulty would be to rear an earlier (i.e., the second) litter for the F_1 evaluation, not the fifth. Thus, this study and the next rat study were designed to test the possibility that rats could be used in an RACB design, and to compare the rearing of a second versus the fifth litters. The design was modified by extending the length of Task 2 from 14 to 16 weeks to accommodate the slightly longer gestation length in rats.

Ethylene glycol monomethyl ether (EGME) was used as a reproductive toxicant whose actions are relatively well defined. Task 1, the dose-range-finding portion of the design, was not conducted, as sufficient data were already available to allow dose-setting for Task 2. Concentration levels for Task 2 were set relatively low, at 0.006, 0.012, and 0.024% weight per volume in drinking water. These concentrations produced consumption estimates of approximately 6, 12, and 26 mg/kg/day. These levels were chosen in an attempt to define a no-effect level for EGME in a rat breeding study and to avoid rank infertility at the top dose. In this study, there were no deaths during Task 2.

During Task 2, there was no treatment effect on the number of litters per pair, although there was a 14% reduction in the number of live pups per litter at the high dose. From a control value of 94%, the proportion of live births declined to 88% at the high dose only. Pup weights adjusted for litter size was increased by 5, 11, and 4% in the low, middle, and high dose groups, respectively.

The last litter was reared to weaning, and weights and survival checked periodically. In the high dose group only, 30 to 40% of the pups died by postnatal day 4. Pup body weights were less than controls only in the high dose females, and only at postnatal days 4 and 7.

To evaluate the performance of rats in a crossover, Task 3 was performed with the control and 0.024% EGME groups. Of the 20 pairs cohabited, only 12 to 14 pairs bore young in each group. In control rats, 63% of cohabited females bore live young, while the proportion was 70% in both crossover groups (treated males × control females, and control males × treated females). There were no treatment-related changes in pup number, weight, or proportion of live births in Task 3.

After the Task 3 young were delivered and assessed, the control and high dose F_0 rats were killed and necropsied. Female body weight was unchanged, but relative liver weight was reduced by approximately 5% in the high dose group. In males, there were no treatment-related alterations in any organ weight, or in epididymal sperm measures.

There were sufficient rats to compose 20 non-sib breeding pairs per group. When the animals were cohabited at 80 ± 10 days of age, 85 to 100% of the rats mated and delivered live young. In the high dose group, there was a 20% reduction in the number of live young. Pup weight adjusted for litter size was increased in the middle and high dose groups by 10 and 9%, respectively.

After the F_2 rats were all delivered and assessed, all rats were killed, and 20 F_1 rats from each group were necropsied. No female organ or body weights were changed by exposure to EGME. Male body weight was reduced by 12% at the high dose, while relative kidney weight at that dose was reduced 6%, and absolute testis weight was increased by 21%. Testis weight was also increased at the middle dose level by 7%. Epididymal sperm density was increased by 18% at the high dose.

In summary, this study showed a decrease in live pups per litter in both generations at the high dose, although neither sex showed adverse effects in the crossover mating. Sufficient offspring were available to conduct the second generation mating, suggesting that the approach of using the fifth litter to generate animals for second generation testing is not limited by the physiology of rat reproduction.

Thus, EGME was clearly toxic to rat reproduction in this design. The overall study length reduction achieved by this design may be offset by the poorer performance of all rats during the crossover, due

to their slightly greater age, and by other considerations presented in the publication comparing these two designs. It appears that either design will work with rats, and indeed, rats are used routinely for RACB studies now, rearing the fifth litter. Two findings of note were the increase in pup weights seen in the low dose in both rat studies and the reduction in epididymal sperm number observed in this study in the low dose F_1 males.

Summary: NTP Reproductive Assessment by Continuous Breeding Study.

NTIS#: **PB90252321**
Chemical: **Ethylene Glycol Monomethyl Ether**
CAS#: **109-86-4**
Mode of exposure: **Drinking water**
Species/strain: **Sprague-Dawley rats**

F_0 generation — Dose concentration →	.006%	.012%	.024%
General toxicity	Male, female	Male, female	Male, female
Body weight	—, —	—, —	—, —
Kidney weight[a]	•, •	•, •	—, —
Liver weight[a]	•, •	•, •	—, ↓
Mortality	—, —	—, —	—, —
Feed consumption	•, •	•, •	•, •
Water consumption	—, —	—, —	—, —
Clinical signs	—, —	—, —	—, —
Reproductive toxicity			
x̄ litters/pair	—	—	—
# live pups/litter; pup wt./litter	—, ↑	—, ↑	↓, ↑
Cumulative days to litter	—	—	—
Absolute testis, epididymis weight[a]	•, •	•, •	—, —
Sex accessory gland weight[a] (prostate, seminal vesicle)	•, •	•, •	—, —
Epidid. sperm parameters (#, motility, morphology)	•, •, •	•, •, •	—, —, —
Estrous cycle length	•	•	•

Determination of affected sex (crossover)	Male	Female	Both
Dose level	—	—	—

F_1 generation — Dose concentration →	.006%	.012%	.024%
General toxicity	Male, female	Male, female	Male, female
Pup growth to weaning	—, —	—, —	—, —
Mortality	—, —	—, —	—, —
Adult body weight	—, —	—, —	↓, —
Kidney weight[a]	—, ↑	—, —	↓, —
Liver weight[a]	—, —	—, —	—, —
Feed consumption	•	•	•
Water consumption	—, —	—, —	↓, ↓
Clinical signs	—, —	—, —	—, —
Reproductive toxicity			
Fertility index	—	—	—
# live pups/litter; pup wt./litter	—, —	—, ↑	↓, ↑
Absolute testis, epididymis weight[a]	—, —	↑, —	↑, —
Sex accessory gland weight[a] (prostate, seminal vesicle)	↑, —	—, —	—, —
Epidid. sperm parameters (#, motility, morphology)	•, •, •	—, —, —	↑, —, —
Estrous cycle length	•	•	•

Summary information	
Affected sex?	Unclear
Study confounders:	None
F_1 more sensitive than F_0?	Unclear
Postnatal toxicity:	Yes

Legend: —, no change; •, no observation; ↑ or ↓, statistically significant change ($p<0.05$); —, —, no change in males or females. [a]Adjusted for body weight.